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EFFECT OF POWDER AND LIQUID PREPARATIONS OF PROBIOTICS ON WHITE SHRIMP (*Litopenaeus vannamei*) GROWTH PERFORMANCE

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Running title: Effect of probiotic preparations on white shrimp growth

ABSTRACT

Research of the effects of application of powder and liquid probiotic on the growth performance of Vannamei shrimp (*Litopenaeus vannamei*) was carried out in Minaloka Jaya shrimp ponds, Grabag District, Purwerojo Regency, Central Java. The purpose of this research was to evaluate powder and liquid probiotics toward white shrimp growth performance. The shrimps were cultivated for 60 days, with three probiotic treatments being used, were commercial liquid probiotics, dosage 10 ml/kg feed, powder probiotics, dosage 10 g /kg feed and liquid probiotic, 10 ml/kg feed. Each probiotic spread over 150,000 vannamei shrimps which cultured in the semi-intensive system, and feeding given four times a day. Probiotics in powder and liquid contain *Lactobacillus fermentum, L. acidophilus, L. plantarum, L. curvatus, Bacillus licheniformis, B. subtilis*, and *B. polimyxa. B. megaterium,B. Coagulans, Pseudomonas putida, Nitrosomonas sp.*,and *Nitrobacter sp.* The research used Randomized Block Design (RBD) with three treatments and five replications. Data were analyzed using analysis of variance with a 95% confidence level and Duncan's Multiple Range Test. Growth of performance was evaluated by specific growth rate (SGR), body length absolute, absolute biomass, and feed conversion ratio (FCR). The results showed that the application of probiotics in powder and liquid could increased growth yield. However that the powder probiotic had better growth performance than commercial liquid probiotics and liquid probiotics. Powder probiotics showed the best results with a specific growth rate of 8.18%, body length absolute of 9.68 cm and absolute biomass of 6.78 g, and feed conversion ratio of 1.93.

Keywords: liquid probiotic, powder probiotic, white shrimp performance

INTRODUCTION

The aquaculture industry is one of industries which has contributed to the greatest economic value for Indonesia. According to data of the Food and Agriculture Organization (FAO) in 2016, Indonesia is one of the countries that has the second largest water production in the world, after China. This fact is supported by statistical data of the Central Bureau of Statistics (BPS) of Indonesia in 2015 that also explained the aquaculture production of Indonesia increased every year (BPS 2015).

One of the leading commodities for brackish water fisheries is white shrimp farming (*Litopenaeus vannamei*) which is used as export commodity. According to Asia Pacific Aquaculture (2015), the production of white shrimps in Indonesia occupied the second position in the United States shrimp market with the amount of 504,000 Metric Ton (MT). White shrimps have benefits compared to tiger shrimps, being higher use of stocking density, so they will produce greater production, be easy to cultivate, and have rapid growth.

Very highly stocking density use will cause the accumulation of residual organic material feed and metabolism in the bottom of the waters, high waste accumulation increases the concentration of various toxic compounds and results in changes in water quality and further increases the growth of opportunistic pathogens and it can disrupt the life of white shrimps (Gao et al., 2016). According to Gao et al., (2016), high stocking densities would result in unfavorable growth, low survival rate, low production, poor water quality, and the emergence of pathogen outbreaks. Therefore probiotics are needed which can work directly on hosts and microbes that work to degrade leftover feed that is not consumed to improve pond water quality. In shrimp aquaculture, probiotics are mainly considered as a disease control agent, because probiotics have been shown to inhibit the growth and proliferation of pathogens, and also stimulate the dominance of microbiota that benefits the host. Probiotics influence growth, nutritional status, and the quality of the rearing environment (Lazado et al., 2015).

Probiotics are defined as living microorganisms, which, when administered in sufficient quantities, will provide health benefits to their host. Probiotics serve to improve the balance of the microbes in the digestive tract of shrimps and can increase the nutritional value of feed and nutrient absorption rate that allows shrimps to achieve maximum growth (Fijan 2014). Probiotic bacteria produce lactic acid and organic acids, reduce the pH environment, and try to prevent the growth of many bacteria. These bacteria produce antimicrobial compounds such as bacteriocin which can be used as natural preservatives (Karami et al., 2017). Application of combination probiotics composed of *Bacillus* sp., *Nitrosomonas* sp., *Nitrobacter* sp. and *Lactobacillus* to Penaeus vannamei ponds produced to the mitigation of nitrogen and phosphate pollution in ponds as shown by the reduction of total phosphorus, total inorganic phosphorus, total nitrogen and total organic carbon (Lazado et al., 2015). Also, Lactobacillus produces antibacterial compounds and increases resistance to attack by pathogens *Vibrio* sp. (Sivakumar et al., 2012).

In the present time the probiotics used in various ponds are commercial liquid probiotic preparation, the popular one is EM-4. Probiotics in liquid form are less efficient in terms of expired stability, storage or packaging, besides the possibility of greater overgrowth of other bacteria compared to powder form (Govender et al, 2013). In addition, it is less practical, especially in remote areas or inter-island. Therefore, powder probiotic preparation is required to solve the transportation problem.

The coating is one way to protect and delivery of probiotics. Encapsulated in polyalcohols of glycerine, sorbitol, mannitol, and prebiotic oligosaccharides inulin, starch and dextrin were effective in the protection of probiotic bacteria during freeze-drying with little change in bacterial cell counts when the bacteria were stored at 4 ° C for five months. Also, probiotics such as have ideal moisture between 2.8-5.6%. While microencapsulation using polysaccharide or protein-based systems is far more effective in the protection of bacteria during freeze-drying and storage as compared to traditional cryo-protection. Polysaccharides, used as prebiotics that protects the probiotic delivered bacteria and produces a synbiotic formulation (Govender et al., 2013).

The provision of powder or encapsulated forms probiotics, which have been widely developed and utilized commercially, is by Spray drying technique using Spray Dryer. In this study, maltodextrin was used as an encapsulation material, because maltodextrin is a source of oligosaccharides for the growth of probiotic bacteria that can facilitate the process of feed nutrient degradation that takes place in the digestive tract of shrimp (Andriani et al, 2017).

Probiotics application is expected to increase the growth of white shrimps, therefore, it is necessary to conduct research about the effect of giving liquid and powder probiotics , and setting probiotic preparations that can improve the growth performance of white shrimps in intensive ponds.

MATERIALS AND METHODS

The research was conducted for three months from October 5 to December 16, 2017, in Minaloka Jaya shrimp pond, Shrimp Club Indonesia (SCI) Grabag Subdistrict, Purwerojo District, Central Java.

The materials used in this research included: a) White prunes PL-9 with length of 7.57 ± 0.8 mm and weight of 70 ± 0.02 mg obtained from PT. Suri Tani Pemuka Hatchery Unit Indramayu; b) Shrimp feed in the form of starter and grower pellets with the brand of '*Pakan Udang'*; c) Commercial probiotics (EM-4), (Powder and liquid probiotic preparations prepared by Laboratory of Microbiology, Universitas Padjadjaran). The laboratorium of Microbiology Universitas Padjadjaran produces probiotics is composed of *L. fermentum*, *L. acidophilus*, *L. plantarum*, *L. curvatus*, B. *licheniformis, B. subtilis,* and *B. polimyxa, B. Coagulans, B. megaterium, Pseudomonas putida*, *Nitrosomonas* sp., and *Nitrobacter* sp. Probiotics are prepared in the lab. microbiology with a cell density of 10 x 10^{10} cfu /g.

The instruments used in this research were a refractometer to measure the salinity of pond waters, pH meter of Lutron brand with type of PH-201 accuracy of 0.1, to measure water pH in the farm pond maintenance, DO meter of Hanna brand, type of HI 9146 (0.1 mg/l) to measure dissolved oxygen, mercury thermometers to measure the temperature of the ponds, the analytical scale (0.01 gram) to measure the white shrimp weight, calipers (0.1 mm) to measure the length of the white shrimp, screen to pick up white shrimp and digital cameras to document every research activity.

This research was experimental with Randomized Block Design (RBD) consisting of 3 treatments and 5 replications. Pond plots for commercial probiotic applications, liquid probiotics and powder probiotics were used as treatment groups.

Procedures

The research was conducted for 60 days using three ponds, each of which used a different treatment. The treatments were plotted as follow: A (area of 0.15 ha), plot treatment B (area 0.18 ha), and treatment plot C (area of 0.18 ha) with stocking density each 150,000 white shrimps per pond plot. Vannamei shrimp cultivation is carried out using a semi-intensive system, which has a maximum area of 1 ha per plot, water depth of 80-100 cm and uses a water mill for aeration.

Feeding was adjusted to the feeding rate (FR), which is 15% of the weight of the vanamei shrimp biomass. The feed dose given follows shrimp growth and is calculated once every week. Probiotic applications from each treatment are:

1. Liquid commercial probiotics (EM-4) mixed with feed at the beginning of stocking until harvest is used as much as 10 ml/kg of feed. Probiotics applied to the water media used a dose of 900 m/ 0.15 ha at the beginning of stocking, then when the shrimp was 30 days old until the harvest was used the treatment was 2.4 liters / 0.15 ha.
2. Liquid probiotics which are produced independently are applied from the beginning of the stocking until the harvest is used as much as 10 ml/kg of feed. Probiotics applied to the waters used a dose of 5.5 liters / 0.18 ha at the beginning of stocking, then when the shrimp was 30 days old until the harvest was used the dose was 2.5 liters / 0.18 ha.
3. Probiotic powder mixed with feed at the beginning of stocking until yield is used as many as 10 g / kg of feed and applied to the waters as much as 5 kg / 0.18 ha at the beginning of stocking, then the dose is 2 kg / 0.18 ha when the shrimp is 30 days old until harvest.

Measurement of shrimp growth parameters is done once a week; each treatment took 3-5 vanamei shrimp at five sampling points.

Measurement of shrimp growth parameters was done once a week; in each treatment 3-5 white shrimps were taken in five sampling points using the screen, then the length and weight were measured using calipers and analytical scale. The calculation of feed conversion ratio (FCR) was performed at the end of the study.

Parameters observed include:

1. Specific Growth Rate (Effendie 1997)

$$G = \frac{(\ln Wt - \ln Wo)}{t} \text{ X } 100\% \qquad (1)$$

Explanation :

G = Growth rate (%)

Wt = Average of fish daily weight at the end of the research (g)

Wo = Average of fish daily weight at the beginning of the research (g)

t = Duration of observation (day)

2. Absolute Length Growth (Effendie 1997)

$$L = L_t - L_o \qquad (2)$$

Explanation :

L = Absolute length growth (cm)

Lt = Average of individuals length at the time refers to t (cm)

Lo = Average of individuals length in the beginning of the research (cm)

3. Absolute Biomass (Effendie 1997)

$$W = W_t - W_o \qquad (3)$$

Explanation :

W = Absolute growth (g)

Wt = Biomass weight at the end of the research (g)

Wo = Biomass weight at the beginning of the research

4. Feed Conversion Ratio (WWF-Indonesia 2014)

$$FCR = \frac{\text{Amount of feed}}{\text{Biomass}} \qquad (4)$$

The parameters of this research supported the water quality measurement including DO, pH, temperature and salinity performed once a week, and ammonia measurement done every two weeks.

Data Analysis

Data were analyzed using analysis of variance with 95% confidence level and Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Specific Growth Rate of White Shrimps

The growth rate is one of the critical parameters for vannamei shrimp cultivation. The success and effectiveness of maintenance time in aquaculture is obtained by evaluating the growth rate of shrimp. Several factors influence the growth rate of shrimp, namely the effectiveness of maintenance time, the effectiveness of feeding, controlling the disease and controlling the conditions and control of the cultivation environment i.e Dissolved Oxygen (DO), carbondioxyde (CO_2), temperature, and salinity (Susilowati et al, 2018).

ANOVA test for data of specific growth rate of vanamei shrimp shows that the data is normal and homogeneously distributed. The results of the variance analysis showed that the treatment is given had a significant effect ($p < 0.05$) on the specific growth rate of shrimp. Significant differences were then tested using Duncan's Multiple Range Test (DMRT). The DMRT results showed that commercial probiotics gave different specific growth rates to the treatment of liquid and powder preparations probiotics.

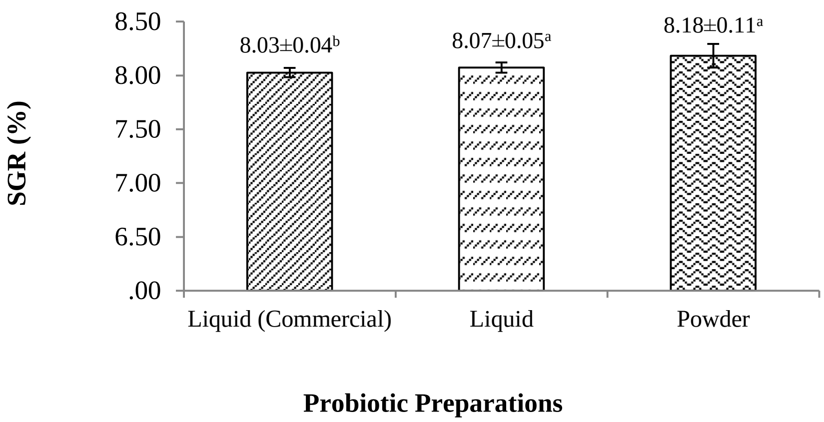

Figure 1 Specific growth rate of Vannamei by the administration of commercial. liquid and powder probiotic on feed

Note: Average values followed by unequal letters showing significant differences ($p < 0.05$)

Figure 1 shows the value of spesific growth rate of white shrimps with commercial, liquid and powder probiotic treatments wich were 8.03%, 8.07% and 8.18%, respectively for 56 days of cultivation. The probiotic powder application produces the highest spesific growth rate of 8.18%. These results were higher than those of Swapna et al. (2015), providing specific growth rate of white shrimps of 4.32% with the administration of powder probiotic Bacillus licheniformis ($10x10^9$ CFU/kg) and Lactobacillus rhamnosus (^{8x109} CFU/kg)) in the feed. According to Sandeepa *et al.*

(2015) the spesific growth rate of white shrimps with the application of probiotics *Lactobacillus* sp. as much as 10% in feed yielded a daily growth rate of 7.16%.

The high specific growth rate in this treatment due to powder probiotic dosage or encapsulated probiotics is a process of coating a core material. In this case, probiotic bacteria as the core material are encapsulated by maltodextrins able to maintain viability and protect probiotic bacteria from damage due to unfavorable environmental conditions. This can be seen from the number of colonies after the encapsulation process (10^{10}-10^{12}). The amount still meets the requirements to be applied to shrimp digestive tracts, which is 10^6-10^8 CFU's/ml (Andriani et al. 2017). The number of bacterial colonies will affect the increased activity of enzymes produced by probiotic bacteria in the digestive tract of shrimps that can improve the digestibility of feed nutrition. Excellent digestibility will optimize the utilization of feed consumed to increase the growth of vannamei shrimps..

The content of probiotic bacteria in powder preparations such as *Bacillus coagulans* is capable of producing amylase and lipase enzymes, and *Bacillus licheniformis* bacteria produce protease enzymes that can increase protein digestibility from feed so as to increase the growth rate of white shrimps. The probiotic bacteria can regulate the microbial environment of the intestine and block the pathogenic microorganisms in the intestine by releasing enzymes (proteases, lipases and amylases) that aid in the digestion of food; with the help of these enzymes, the nutrients in feed will be more easily absorbed by the shrimp digestive organs so that shrimps can grow maximally (Salz et al., 2019).

Absolute Length Growth of Vannamei Shrimp

ANOVA on absolute length growth and absolute biomass data of Vannamei shrimps showed normal and homogeneously distributed data. The results of variance analysis on absolute length growth and absolute biomass growth showed that the treatment given significantly affected ($p < 0.05$) the growth of test shrimps. The DMRT results showed that commercial probiotics gave different absolut length growth on the treatment of liquid and powder probiotics preparations.

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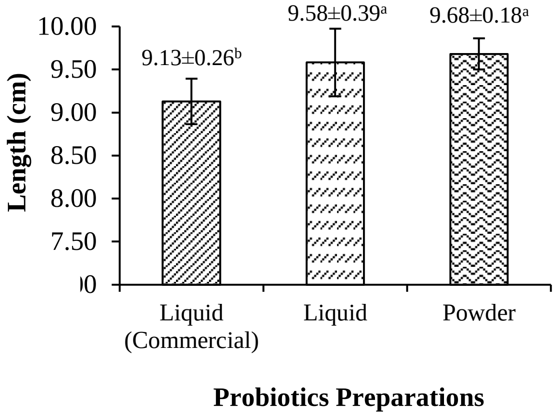

Figure 2 Absolute length growth of Vannamei shrimp in the provision of probiotic of commercial, liquid probiotic and probiotic powder on feed

Note: Average values followed by unequal letters showing significant differences ($p < 0.05$)

Figure 2 shows the absolute growth obtained in giving a powder probiotic, with a value of 9.68 cm for 56 days, which is significantly different from commercial probiotics and liquid probiotics respectively of 9.13 cm and 9.58 cm. Furthermore, absolute biomass growth of white shrimps showed that the powder probiotic treatment resulted in the highest biomass growth, which was 6.78 g compared to commercial and liquid probiotic treatments of 6.20 g and 6.37 g (Figure 3), respectively.

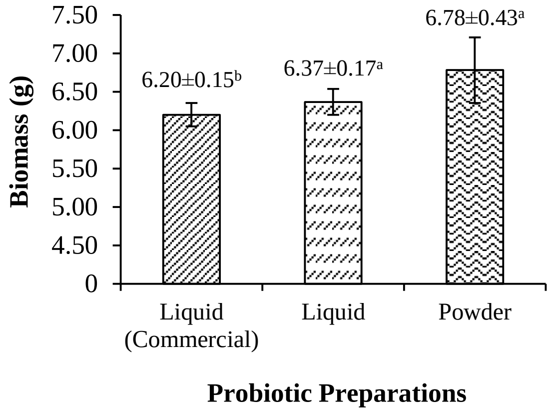

Figure 3 Absolute biomass growth of Vannamei shrimp on the administration of commercial, liquid, and powder probiotic on feed.

Note: Average values followed by unequal letters showing significant differences ($p < 0.05$)

Figure 4 and Figure 5 show the length growth and average biomass of white shrimp during cultivation. These results indicate that the process of adding to the length and biomass of the shrimp runs during cultivation. According to Fendjalang et al. (2016), shrimp growth is influenced by heredity, gender, age, density, parasites, and diseases and the ability to use feed. Weight gain is influenced by feed consumption because feed consumption determines the input of nutrients into the body for growth and metabolism. Length increase and biomass indicate that the application of probiotics can improve nutrient utilization by shrimp.

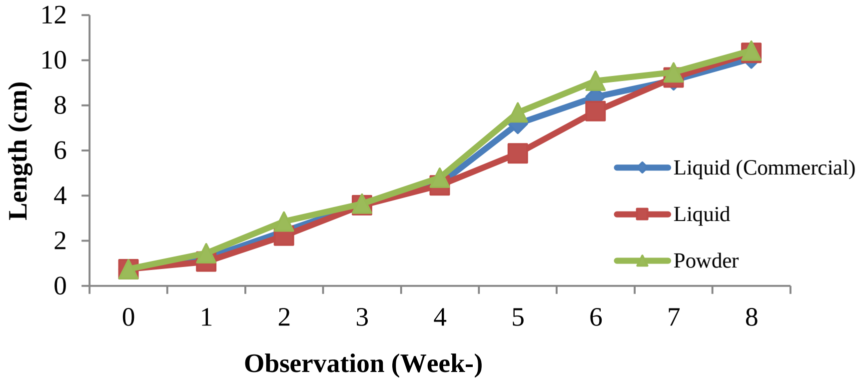

Figure 4 Average length growth of Vannamei with commercial, liquid and powder probiotic applications on feed during cultivation

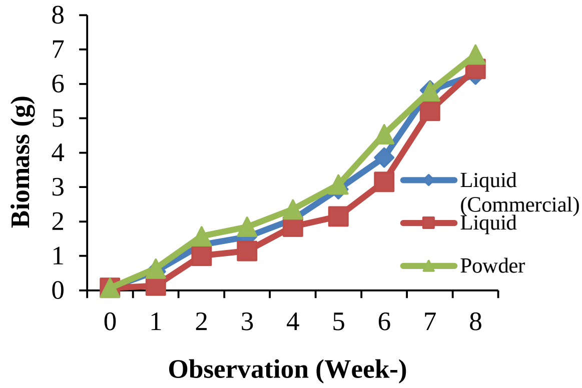

Figure 5 Average of biomass growth of Vannamei with commercial, liquid and powder probiotic applications on feed during cultivation

Length growth and biomass in the treatment provided by this powder-stock probiotic showed the role of different probiotic compositions in white shrimp growth. The role given is presumably due to the contribution of enzymes to digestion that cause shrimps to digest feed better, so that more nutrients are absorbed by the shrimp digestive tract and the growth will increase. Enzymes that are thought to play a role in helping the shrimp larvae digestion are proteases and amylases. According to Widanarni *et al*. (2012) *Bacillus megaterium* bacteria play an effective role in the digestive process by producing extracellular enzymes such as proteases, carbohydrolases and lipases that can maintain beneficial bacterial growth in the shrimp tract, thereby increasing growth.

The Pseudomonas putida consortium contained in probiotic preparations has a mechanism to produce several exogenous enzymes to digest feed such as amylase, protease, lipase, and cellulose.

Also, protease enzymes are produced by Bacillus licheniformis, which can increase the digestibility of feed proteins. The exogenous enzyme will help endogenous enzymes in the host to hydrolyze feed nutrients. The availability of enzymes produced by probiotics will increase the availability of nutrients that are readily absorbed from the digestive tract to enter the blood vessels and will be circulated to all parts of the body and tissues needed for further metabolic processes. According to Rajikkannu *et al.* (2015) the provision of *Bacillus pumilus* probiotic bacteria with proper concentration and dosage can increase the number of hemoglobin; this is believed to be one indicator of increasing shrimp ability in supplying nutrients to the body and repair system and increasing the growth of white shrimp. The higher the nutrient of the digestible feed, the greater posibility of nutrients being exploited by the shrimp for its growth will be.

Feed Conversion Ratio

Feed conversion ratio is a description of effectiveness level of feed given to shrimp growth response obtained. FCR is an indicator of how far the feed given can be utilized by shrimp to raise 1 kg of shrimp weight. The conversion rate results of white shrimp feed (Fig. 6) from each treatment, namely commercial probiotic liquid preparation, probiotic liquid dosage and probiotic powder dosage were 2.46, 1.83 and 1.93, respectively.

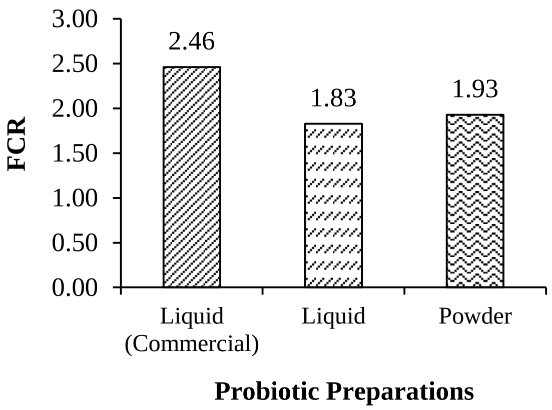

Figure 6 Feed convertion ratio of Vannamei with commercial, liquid and powder probiotic applications on feed during cultivation

Probiotic treatment of liquid preparations showed the lowest FCR value compared to other treatments. The results were not much different from the research of Susilowati *et al.* (2017) which resulted in the value of FCR of white shrimp equal to 1.42 by adding probiotic liquid preparation in pond plot. According to Tahe *et al.* (2015) generally, the value of FCR less than 2 is still declared good in semi-intensive shrimp farming. Judging from the value of FCR obtained, then pond plots with probiotic treatment liquid and powder preparations are able to produce good feed conversion to support the successful cultivation of white shrimps.

Low feed conversion is thought due to the role of a consortium of different probiotic bacteria added to pond waters and into feed as well. The added probiotic bacteria will increase the activity of digestive enzymes significantly in the shrimp body, thereby increasing the nutrient digestion and resulting low FCR value. According to Valsamma *et al*. (2014), probiotic treatment increased the activity of amylase and trypsin enzymes in shrimp. According to Tahe *et al.* (2015) the addition of probiotic bacteria in the diet can improve the growth performance and feed conversion ratio; this is related to the cellulose and amylase enzymes produced by *Bacillus* sp. The use of probiotics in shrimp farming can improve nutrient digestibility, feed efficiency and tolerance to stress (Cruz *et al.* 2012).

Optimal in feed digesting and absorbing will result in low feed conversion value to change the feed into meat in optimal time. Probiotic bacteria that can help in feed absorption and shrimp growth include the genus Bacillus (Rajikkannu et al. 2015). In the powder probiotics, there are several species of the genus Bacillus, among which are *Bacillus circulans, B. subtilis, B. coagulan, B. amyloliquefaciens, B. pumilus, B. licheniformis,* and *B. megaterium*. *Bacillus bacteria* will increase feed absorption and subsequently play a role in increasing the weight of white shrimps and low feed conversion. Increased intake of feed is caused by the balance of microbes in the digestive tract of shrimps.

In addition, there are *Lactobacillus* sp. which are lactic acid-producing bacteria that serve to increase the enzyme's effectivity and help digest the food (Buruina *et al.* 2014). Increased fat metabolism due to increased enzyme activity, one of which is lipase, will increase the utilization of fat-rich diet as an energy source so the conversion of protein to meat will be more optimal (Dhanalakshmi *et al.* 2015). The activity of bacteria in the digestion will change rapidly when there are beneficial microbes entering through the feed or water causing changes in the balance of existing bacteria with bacteria entering in the digestive tract of shrimps, so the process of feed protein absorption will be more optimal. Based on the data above, the application of liquid and powder probiotic preparations with several beneficial bacterial consortiums contained are able to improve the efficiency of feed which is marked by the low value of feed conversion of white shrimps produced.

Water Quality Parameters

Water quality plays an important role in supporting the life and growth of white shrimps. Observations of several water quality parameters including temperature, dissolved oxygen, salinity, pH, alkalinity and ammonia in all treatments during the study are presented in Table 1. Water quality has a significant impact on shrimp health.

Table 1 White Shrimp Aquaculture FarmingWater Quality

Parameters	Probiotic Preparations			
	Liquid (Commercial)	Liquid	Powder	Tolerant[a]
Temperature (^{o}C)	29.6 - 31.6	29.9 – 32.4	29 - 31.8	20 - 35^{o}C
DO (mg/L)	6.57 - 8.47	5.87 - 7.47	6 - 7.2	>3 mg/L
Salinity (ppt)	9.6 - 19.63	13.4 - 19.8	9.87 - 19.7	0-35 < 35 ppt
Alkalinity (mg/L)	110 - 142	108 - 132	62 - 140	100 mg/L
Ph	7.7 - 8.4	7.73 - 8.33	6.7 - 8.37	7 - 8.5
Ammonia (mg/L)	0.1023 - 0.259	0.001 - 0.191	0.019 - 0.180	0.1 - 0.5 (mg/L)

[a]WWF-Indonesia 2014

Water quality fluctuations in maintenance media can result in low growth rate, syntax, and frequency of skin change, and harmful bacteria increase. The water quality of maintenance media during the study remained within a reasonable range for the growth and stability of white shrimp, but in the probiotic powder treatment, the pH and alkalinity values were less than optimal.

Table 1 shows the temperature values in commercial, liquid, and probiotic powder treatments, i.e., 29.6-31.6°C; 29.9-32.4oC; and 29-31.8oC. The range is still within the optimal range for the life of white shrimps. The optimal temperature for white shrimp cultivation ranges from 28-32oC with tolerance range 20-35oC (WWF-Indonesia 2014). According to Tahe et al. (2015), the optimal temperature reaches from 27-32°C. If the temperature is higher than the tolerance rate, then the metabolism in the body of the shrimp will take place quickly so that the demand for dissolved oxygen increases.

The measurement results of dissolved oxygen content (DO) in the white shrimp culture medium during the maintenance of each treatment were 6.57-8.47 mg/L; 5.87-7.47 mg/L; and 6-7.2 mg/L. The dissolved oxygen content in water is a critical factor for shrimp health; with the DO value obtained then the value is still in the optimal category (minimum tolerance of 3mg/L according to WWF-Indonesia 2014). According to Tahe et al. (2015) reports that minimum DO for shrimp health is 3.0 mg/L and DO <2.0 mg/L would cause death to shrimp.

Furthermore, the salinity value obtained during the study of each commercial, liquid and powder probiotic treatment was 9.6-19.63 ppt; 13.4-19.8 ppt; and 9.87-19.7 ppt. The salinity is in the optimal conditions for the life of white shrimps; salinity tolerance values are 0-35, <35 ppt (WWF-Indonesia 2014), The values of alkalinity during cultivation of white shrimps from each treatment of commercial probiotics, liquid and powder probiotics obtained were 110-142 mg/L, 108-132 mg/L, and 62-140 mg/L, respectively. According to WWF-Indonesia (2014) the tolerance value of alkalinity content in a medium of cultivation is 100 mg/L. The treatment of powder probiotics obtained the lowest alkalinity value of 62 mg/L due to weather factors of fairly high rainfall intensity. Rain water is water that has a pH value of about 5; in the presence of rain it will decrease the pH and total alkalinity in the pond, so that with low alkalinity value, the buffer (water resistance) to water pH fluctuations is very low, resulting in shocks pH with quite low values.

The measurement of pH value from each treatment of commercial, liquid and powder probiotics were 7.7-8.4, 7.73-8.33, and 6.7-8.37, respectively. According to WWF-Indonesia (2014) the optimum pH value is 7.5-8 with a tolerance of 7-8.5, It is due to the low alkalinity value which can cause the decrease of pH content in cultivation media. In the cultivation of white shrimps with the application of probiotics, the pH range value is very important; the pH value range affects the osmotic pressure between the maintenance environment and the shrimp body. If the pH value range is not optimum then the shrimps will require more energy to do the osmoregulation process and energy that should be earmarked for growth is then diverted for the process of adapting to fluctuating pH changes.

Furthermore, the ammonia measurements contained in the white shrimp culture medium from each treatment were 0.1023-0.259 mg/L; 0.001-0.191 mg/L; and 0.019-0.180 mg/L. According to WWF-Indonesia (2014) tolerance of ammonia content in a shrimp culture medium ranges from 0.1 to 0.5 mg/L. This result indicates that water ammonia levels on the maintenance media can still be tolerated by white shrimps.

CONCLUSION

The application of probiotic liquid and powder preparations can improve the growth performance of white shrimps in intensive scale cultivation. Provision of probiotic preparations in powder and liquid forms provide a relatively equal growth increase in support of successful shrimp farming activities. Powder probiotic preparation is the best probiotics that can increase the growth of white shrimps, which can produce daily growth rate of 8.18%, absolute growth of 9.68 cm and absolute biomass of 6.78 g, and feed conversion ratio of 1.93.

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REFERENCES

Andriani, Y, R Safitri and F Shaiyanne. 2018. Vannamei Shrimp (*Litopenaeus vannamei*, Boone, 1931) Performance with Bacillus and Lactobacillus Probiotic Formulation Consortium in Dry Preparation. Res. J. Chem. Environ. Special Issue August (2018):307-312.

Andriani Y, Safitri R., Kanza AA, Rustama, MM. 2017. Karakterisasi Bacillus dan Lactobacillus yang Dienkapsulasi dalam Berbagai Bahan Pembawa untuk Probiotik Vannamei (*Litopenaeus vannamei* Boone, 1931). J. Perikanan dan Kelautan 7(2):150.

Asia Pasific Aquaculture [Internet]. 2015. Akuakultur Indonesia di Level Asia Pasifik. [cited 2017 June 18]. Available from: http://www.djpb.kkp.go.id.

Badan Pusat Statistik [Internet]. 2015. Produksi Perikanan Menurut Subsektor. [cited 2017 May 16] Available from: http://www.bps.go.id/linkTabelStatis/view/id/1711.

Buruina CT, Profir AG, Vizireanu C. 2014. Effects of Probiotic Bacillus species in Aquaculture - An Overview. The Annals Of The University Dunarea De Jos Of Galati Fascicle. J. Vi-Food Technology 38(2):9-17.

Cruz PM, Ibanez HOA, Monroy, Saad HCR. 2012. Use of Probiotics in Aquaculture. Review Article. International Scholarly Research Network ISRN Microbiology. 2012 : 13 p.

Dhanalakshmi G, Reniprabha A, Chandarakala A. 2015. Studies on The Effcet of Commercial Probiotic Application in The Growth of The Fish. International Journal of Advanced Research 3(8):708-712.

Effendie I. 1997. Biologi Perikanan. Jakarta: Yayasan Pustaka Nustama.

Fendjalang SNM, Budiardi T, Supriyono E, Effendi I. 2016. Production of Vaname Shrimp (*Litopenaeus vannamei*) on Floating Net with Different Spreed in Kepulauan Seribu Strait. J. Ilmu dan Teknologi Kelautan Tropis 8(1):201-214.

Fijan S.2014. Microorganisms with Claimed Probiotic Properties: An Overview of Recent Literature. International Journal Environment Res. Public Health 11:4745.

Food and Agriculture Organization [Internet]. 2016. Fishary for Statistical Time Series. [cited 2017 May 16]. Available from: http://www.fao.org/fishery/statistics/software/fishstatj/en.

Gao, Y., Z. He1, H. Vector, B. Zhao1, Z.Li, J. He, J.Y. Lee, and Z. Chu. 2017. Effect of Stocking Density on Growth, Oxidative Stress and HSP 70 of Pacific White Shrimp Litopenaeus vannamei. Turkish Journal of Fisheries and Aquatic Sciences 17: 877-884

Govender, M. Yahya EC,Pradeep K, Lisa C. du Toit, Sandy van Vuuren, and Viness Pillay. 2014. A Review of the Advancements in Probiotic Delivery: Conventional vs. Non-conventional Formulations for Intestinal Flora Supplementation. AAPS PharmSciTech 15(1): 29–43.

Karami, S., M. Roayaei, H.Hamzavi, M. Bahmani, H.H-Azar, M., Leila, and M.Kopaei¨ 2017. Isolation and identification of probiotic *Lactobacillus* from local dairy and evaluating their antagonistic effect on pathogens**.** Int J Pharm Investig. Jul-Sep; 7(3): 137–141. doi: 10.4103/jphi.JPHI_8_17

Lazado, C C, J I. Lacsamana and CM.A. Caipang. 2015. Mechanisms of probiotic actions in shrimp: Implications to tropical aquaculture. Biotechnological Advances in Shrimp Health Management in the Philippines, 2015: 89-114.

Luthfi MZ, Sri R, Tita E. 2017. Analisa Kelayakan Usaha Budidaya Polikultur Udang Windu (*Penaeus monodon*) Dan Ikan Koi (*Cyprinus carpio*) Di Desa Bangsri, Kabupaten Brebes. Jurnal Sains Akuakultur Tropis 1:62-71

Nandhini, RS, E Sahana and B Dhanakkodi. 2015. Beneficial and destructive effects of probiotics in aquaculture systems-A review. Intl. J. Fish and Aqua Stud 2015; 2(3): 153-159

Paul, P., A Rahman, M Hossain, S Islam, S Mondal, ; M Haq. 2016. Effect of Stocking Density on the Growth and Production of Freshwater Prawn (*Macrobrachium rosenbergii*). Intl. J. of Fish and Aq.Sci. 6(1):77-86

Sandeepa MG, Ammani K. 2015. Effect of Probiotic Bacterium on Growth and Biochemical Parameters of Shrimp *Litopenaeus vannamei*. International Journal of Recent Scientific Research 6(2):2872.

Sivakumar, N. M Sundararaman and G Selvakumar. 2012. Probiotic effect of Lactobacillus acidophilus against vibriosis in juvenile shrimp (*Penaeus monodon*). Afr. J. of Biotechnol 11(91): 15811-15818

Susilowati T, Herawati VE, Basuki F, Yuniarti T, Rachmawati D, Suminto. 2017. Performa Produksi Udang Vaname (*Litopenaeus vannamei*) yang dibudidayakan pada Tambak Sistem Semi Intensif dengan Aplikasi Probiotik. J. PENA Akuatika 16(1).

Susilowati T, Desrina, J Hutabarat, S Anggoro, M Zainuri, Sarjito, F Basuki and T Yuniarti.2018. The effects of season, aeration and light intensity on the performance of pacific whiteleg shrimp (*Litopenaeus vannamei*) polycultured with seaweed (*Gracilaria verrucosa*). IOP Conf. Series: Earth and Environmental Science 137 (2018):1-7

Swapna B, Venkatrayulu CH, Swathi AV. 2015. Effect of probiotic bacteria *Bacillus licheniformis* and *Lactobacillus rhamnosus* on growth of the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931). European Journal of Experimental Biology 5(11):31-36.

Tahe S, Suwoyo HS, Fahrur M. 2015. Aplikasi Probiotik Rica dan Komersial pada Budidaya Udang Vaname (*Litopenaeus vannamei*) Pola Intensif. Proccedings: 2015: Forum Inovasi Teknologi Akuakultur p. 435-445.

Valsamma, J., Haseeb, M., Ranjit, S., Anas, A. and Brigh, I. S. 2014. Shrimp Production under Zero Water Exchange Mode Coupled with Bioremediation and Application of Probiotics. J. of Fisheries International 9(1):5-14.

Widanarni, D., Yuniasari, Sukenda, and Ekasari, J. 2012. Nursery Culture Performance of *Litopenaeus vannamei* with Probiotics Addition and Different C/N Ratio under Laboratory Condition. J. of Biosciences 17(3):115-119.

WWF-Indonesia. 2014. Budidaya Udang Vaname Tambak Semi Intensif dengan Instalasi Pengelolaan Air Limbah (IPAL). Jakarta.